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Abstract

Foxa2 (Hnf3beta) is a winged-helix/forkhead transcription factor that regulates gene expression in the liver, pancreatic islets and adipocytes. It is required for the maintenance of glucose and lipid homeostasis. Hyperinsulinemia-mediated inactivation of Foxa2 by nuclear exclusion has recently been implicated in the development of liver steatosis and insulin resistance in three animal models of diabetes. These abnormalities were cured by adenovirus-mediated expression of a constitutively active form of Foxa2 containing a mutated T156 phosphorylation site, which increases fatty acid oxidation and reduces its biosynthesis. Accordingly, the prevention of phosphorylation of Foxa2 was suggested as a pharmacological target for the treatment of obesity and diabetes.

Reference

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Does chasing selected ‘Fox’ to the nucleus prevent diabetes?

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Foxa2 (Hnf3β) is a winged-helix/forkhead transcription factor that regulates gene expression in the liver, pancreatic islets and adipocytes. It is required for the maintenance of glucose and lipid homeostasis. Hyper-insulinemia-mediated inactivation of Foxa2 by nuclear exclusion has recently been implicated in the development of liver steatosis and insulin resistance in three animal models of diabetes. These abnormalities were cured by adenovirus-mediated expression of a constitutively active form of Foxa2 containing a mutated T156 phosphorylation site, which increases fatty acid oxidation and reduces its biosynthesis. Accordingly, the prevention of phosphorylation of Foxa2 was suggested as a pharmacological target for the treatment of obesity and diabetes.

The obesity and diabetes pandemic

Overweight and sedentary lifestyles in modern society contribute to the development of obesity and diabetes. Type 2 diabetes, which affects an estimated 6% of the population in industrialised nations, is characterized by insulin resistance and abnormal insulin secretion from pancreatic β-cells [1]. The accumulation of lipids in the liver and islets has been suggested as one of the causal factors. An elegant study by Stoffel and colleagues [2] has enhanced our understanding of the molecular mechanisms underlying the development of fatty liver, insulin resistance and diabetes. Here, we highlight these findings and place them into the larger context of glucose and lipid homeostasis.

Foxa2 inactivation causes liver steatosis and insulin resistance

The cytoplasmic localization and inactivation of a winged-helix/forkhead transcription factor, Foxa2 [hepatocyte nuclear factor-3β (Hnf3β)], has been demonstrated in the liver of three well-characterized insulin-resistant mouse models, including ob/ob, lipoatrophic ap2–nSrebp–1c and high-fat-induced obese mice [2]. The authors suggested that chronic hyperinsulinemia in type 2 diabetes caused the inactivation of Foxa2 by nuclear exclusion, thereby deteriorating hepatic lipid accumulation and insulin resistance through increased fatty acid biosynthesis and reduced β-oxidation. However, insulin-mediated Foxa2 compartmentation remains to be confirmed in the liver and extended to other insulin target tissues.

Intriguingly, Stoffel and colleagues [2] also show the nuclear retention of another forkhead family member, Foxo1, in hepatocytes of these insulin resistant or diabetic animals. Similar to Foxa2, Foxo1 is an effector of insulin
signaling in hepatocytes [3], pancreatic islets [4] and adipocytes [5]. Foxo1 promotes gluconeogenesis in the liver during fasting and is inactivated upon feeding through insulin-signaling-mediated phosphorylation and nuclear exclusion [3]. The differential subcellular localization of Foxa2 and Foxo1 in the hepatocytes of insulin-resistant mice is explained through the following mechanisms. First, Foxa2 is more sensitive to insulin signaling than is Foxo1, because Foxa2 is phosphorylated through both insulin receptor substrate-1 (IRS1) and IRS2 pathways, whereas Foxo1 is inactivated only by IRS2 (Figure 1) [2]. Second, IRS2 signaling is presumably defective in these insulin-resistant animals [2]. Consequently, the nuclear retention of Foxo1 should contribute to the increased hepatic glucose production in these mice.

**Foxa2 activation improves hepatic lipid metabolism and insulin sensitivity**

Another important finding of the Stoffel et al. study [2] was that the constitutive activation of Foxa2 in the liver of insulin-resistant mice through gene manipulation resulted in decreased hepatic triglyceride content, increased hepatic insulin sensitivity, reduced glucose production, normalized plasma glucose and significantly lowered plasma insulin. This was achieved by the adenovirus-mediated expression of a nuclear localized and active mutant (T156A) of Foxa2, which is resistant to insulin phosphorylation. Gene-chip expression profiles revealed that hepatic Foxa2 activation induces the expression of genes implicated in triglyceride degradation, fatty acid transport, mitochondrial and peroxisomal β-oxidation, ketogenesis and glycolysis. The Foxa2-mediated improvement of hepatic steatosis and insulin action is also partly explained by the upregulation of peroxisome proliferator-activated receptor-γ (Ppar-γ) [6], Hnf4α [7] and uncoupling protein-2 (Ucp-2) and Ucp-3 [8], in addition to the downregulation of fatty acid synthase (Fas) and stearoyl-CoA desaturase-1 (Scd-1) [9]. It has recently been reported that adenovirus-mediated hepatic overexpression of Ucp-1 dissipates energy stores and reverses high-fat-diet-induced obesity and diabetes [8]. Moreover, Scd-1-deficient mice are resistant to diet-induced obesity through increased insulin sensitivity, fatty acid oxidation and energy expenditure [9]. Therefore, Foxa2 might function by targeting multiple genes in the liver.

Stoffel and colleagues [2] have also reported that the livers of Foxa2+/− mice fed a high-fat diet display a pronounced reduction in β-oxidation and ketogenesis. This is associated with increased levels of plasma triglycerides and free fatty acid and twofold higher hepatic glucose production in the haploinsufficient Foxa2 mice compared with wild-type littermates. These results suggest that Foxa2 has a predominant role in the regulation of hepatic lipid metabolism rather than gluconeogenesis. By contrast, Foxo1 acts to regulate the expression of genes encoding enzymes of gluconeogenesis through a direct interaction with Ppar coactivator-1α (Pgc-1α), which is induced in the liver during fasting (Figure 1) [3].

The homozygous Foxa2-null mutation in mice leads to embryonic lethality because Foxa2 is required for the formation of the foregut, from which liver and pancreas

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**Figure 1.** The regulation and function of Foxo and Foxa transcription factors. Foxo3a, Foxo4, Foxo1 and Foxa2 are activated during fasting and inactivated by insulin signaling, through phosphorylation and nuclear exclusion. Foxo transcription factors are also activated by caloric restriction through Sir2/SIRT1-mediated deacetylation. The activation of Foxo transcription factors is implicated in cell-cycle arrest and the prevention of oxidative stress, thereby increasing longevity. Foxo1 activation predominantly promotes gluconeogenesis through a direct interaction with PGC-1α, whereas Foxa2 acts mainly on hepatic fatty acid oxidation. Both Foxo1 and Foxa2 inhibit adipocyte differentiation. Foxa1 and Foxa2 regulate glucagon expression and the latter is required for α-cell differentiation. Foxa3 also induces the expression of gluconeogenic genes. Therefore, Foxo and Foxa transcription factors belong to a group of genes that have evolved for adaptation to starvation. Red lines denote inhibitory effects whereas green arrows represent stimulatory pathways.

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are derived [10]. However, the targeted deletion of Foxa2 in the liver reveals its nonessential role in maintaining the differentiated state of adult hepatocytes under physiological conditions [11]. The study by Stoffel and colleagues [2] enables reevaluation of the function of hepatic Foxa2 and demonstrates that it has an essential role in maintaining lipid and glucose homeostasis in diabetes and during fasting [2]. In fact, Costa and co-workers [12] had already demonstrated that the hepatic overexpression of Foxa2 in transgenic mice promoted the expression of genes directing fatty acid β-oxidation.

**Foxa2 and Foxo1: ancient genes evolved for adaptation to starvation**

Mammalian evolution has resulted in genotype selection for the survival of famine. Some of these selected genes might have become pathogenic factors, causing obesity and diabetes in individuals as a result of overnutrition and sedentary lifestyle. The insulin-signaling-mediated threonine phosphorylation site in Foxa2 is well conserved from insects to mammals [13], suggesting that Foxa2 is an ancient gene that evolved to regulate energy homeostasis (Figure 1). Indeed, hepatic Foxa2 is activated during fasting, thereby providing energy through increased fatty acid oxidation [2]. In addition, Foxa2 is a negative regulator of adipocyte differentiation, resulting in reduced energy storage [14]. However, the insulin-mediated regulation of subcellular translocation and inactivation of Foxa2 has not been investigated in preadipocytes. Moreover, Foxa2 is required for the differentiation of pancreatic β-cells [15], secreting glucagon in response to fasting-induced hypoglycemia. Furthermore, β-cell-specific disruption of Foxa2 in mice results in hyperinsulinemic hypoglycemia, suggesting that it regulates β-cell function and insulin secretion (Figure 2). Whether insulin phosphor-ylates and inactivates β-cell Foxa2 remains unknown.

In contrast to Foxa2, two other Foxa members, Foxa1 (Hnf3α) and Foxa3 (Hnf3γ), are not phosphorylated by insulin-signaling cascades [13]. Foxa1 is a potent trans-activator mediating glucagon gene expression [16,17], whereas Foxa3 regulates glucose homeostasis during a prolonged fast through the maintenance of hepatic Glut2 and gluconeogenic gene expression [18,19].

At least three additional factors of the more than 100 forkhead gene family members, including Foxo1, Foxo3a, and Foxo4, contain consensus phosphorylation sites for insulin signaling. They are also inactivated by insulin, similar to Foxa2 [13,20]. Increased longevity has been reported in Caenorhabditis elegans that are deficient for insulin signaling cascades and mice with white-adipose-tissue-specific ablation of the insulin receptor [19]. Caloric restriction also causes similar increased longevity through Sir2/SIRT1-mediated deacetylation and the subsequent activation of the Foxo transcription factors, inducing genes involved in cell growth arrest and anti-oxidative stress (Figure 1) [20]. In particular, Foxo1 has an essential role in maintaining glucose homeostasis during fasting. In addition to the aforementioned function as a master regulator of gluconeogenesis in the liver, its inhibitory effects on adipocyte differentiation [5] and pancreatic β-cell function [4] (Figure 2) also support the notion that Foxo1 belongs to a group of genes evolved for adaptation to starvation. It also represents a typical causal factor for the development of obesity and type 2 diabetes, evidenced by numerous studies showing that Foxo1 halploinsufficiency prevents insulin resistance and diabetes in several mouse models, including Insr<sup>+</sup>− [21], Irs2<sup>−−</sup> [4] and high-fat-diet-fed mice [5].

**FOXa2: a susceptibility gene for diabetes or hyperinsulinemic hypoglycemia?**

Foxa2 has been suggested as one of the upstream regulators of Pdx-1, which is required for β-cell differentiation [22]. The study by Stoffel and colleagues [2] also points to Foxa2 as a candidate gene for diabetes. However, the association of mutations and polymorphisms in the FOXA2 gene with type 2 diabetes has not yet been confirmed [23].

Alternatively, mutations of FOXA2 could contribute to hyperinsulinemic hypoglycemia. Pancreatic β-cell-specific deletion of Foxa2 in mice results in hyperinsulinemic hypoglycemia [11,24]. This is associated with reduced expression of the ATP-sensitive K<sup>−−</sup>-channel subunits Kir6.2 and Sur1 and short-chain 3-hydroxyacyl-CoA dehydrogenase (Schad) [11,24]. Human loss-of-function mutations in these three genes are linked to persistent

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**Figure 2.** Contributions of Foxo1 and Foxa2 activities to β-cell function. Foxo1 suppresses Pdx-1-mediated β-cell gene expression and inhibits β-cell proliferation. By contrast, Foxa2 acts as an upstream transactivator of Pdx-1 transcription during embryonic development and possibly in adult β-cells. However, Foxa2 reduces insulin secretion by regulating the K<sub>ATP</sub>-channel subunits Sur1 and Kir6.2 and the fatty acid metabolic enzyme Schad, and by inhibiting hexokinases. Red lines denote inhibitory effects whereas green arrows represent stimulatory pathways.
hyperinsulinemic hypoglycemia of infancy [25,26]. Conversely, the gain of function following Foxa2 overexpression in rat insulinoma INS-1 cells suppresses insulin secretion [27]. This is in line with the requirement for Foxa2 in the regulation of glucagon gene expression and pancreatic β-cell differentiation [15].

Foxa2: a pharmacological target for the treatment of obesity and type 2 diabetes?

Stoffel and colleagues [2] also suggest that pharmacological intervention to inhibit Foxa2 phosphorylation could be an effective treatment for type 2 diabetes. Before the pharmaceutical industry can be convinced to focus on Foxa2, the following problems should be addressed: (i) identify the target factor or factors that are implicated in the dephosphorylation or activation of Foxa2 during fasting; (ii) the tissue specificity of potential inhibitors of Foxa2 phosphorylation. Is Foxa2 in islet β-cells phosphorylated in a similar way as in the liver? If the answer is yes, this could be the molecular mechanism underlying increased insulin secretion in compensation for insulin resistance. It is possible that a reduction of hyperinsulinemia could be beneficial in obesity similar to weight reduction and exercise; and (iii) target specificity; for example, do the agents designed to inhibit Foxa2 phosphorylation also activate Foxo1 function?

Therefore, Foxa2 is a theoretically interesting, albeit practically challenging, target for the development of compounds aimed at treating obesity and diabetes.

Concluding remarks

Stoffel and colleagues [2] recently reported that hyperinsulinemia-mediated phosphorylation and nuclear exclusion of Foxa2 were associated with the development of liver steatosis and insulin-resistance in type 2 diabetes. Adenovirus-mediated expression of a phosphorylation-site-mutated Foxa2 in the liver of insulin-resistant mice resulted in decreased hepatic triglyceride content and increased hepatic insulin sensitivity. Accordingly, interfering with the phosphorylation of Foxa2 was suggested as a pharmacological target for the treatment of obesity and diabetes. However, many questions remain to be addressed. For example, the nuclear exclusion and inactivation of Foxa2 by insulin should be confirmed in the liver and extended to adipocytes and islets. In addition, the more pronounced sensitivity to insulin signaling of Foxa2 compared with Foxo1 should be reevaluated. Moreover, the Foxa2 gene has been evolved for adaptation to starvation rather than as a native target for diabetes therapy. Therefore, tissue and target specificity are challenging topics for pharmacological approaches. The mechanism of activation or dephosphorylation of Foxa2 during fasting also remains to be resolved.

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